

NTP TECHNICAL REPORT
ON THE
TOXICOLOGY AND CARCINOGENESIS
STUDIES OF
DIETHANOLAMINE
(CAS NO. 111-42-2)
IN F344/N RATS AND B6C3F₁ MICE
(DERMAL STUDIES)

NATIONAL TOXICOLOGY PROGRAM
P.O. Box 12233
Research Triangle Park, NC 27709

July 1999

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U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
National Institutes of Health

FOREWORD

The National Toxicology Program (NTP) is made up of four charter agencies of the U.S. Department of Health and Human Services (DHHS): the National Cancer Institute (NCI), National Institutes of Health; the National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health; the National Center for Toxicological Research (NCTR), Food and Drug Administration; and the National Institute for Occupational Safety and Health (NIOSH), Centers for Disease Control and Prevention. In July 1981, the Carcinogenesis Bioassay Testing Program, NCI, was transferred to the NIEHS. The NTP coordinates the relevant programs, staff, and resources from these Public Health Service agencies relating to basic and applied research and to biological assay development and validation.

The NTP develops, evaluates, and disseminates scientific information about potentially toxic and hazardous chemicals. This knowledge is used for protecting the health of the American people and for the primary prevention of disease.

The studies described in this Technical Report were performed under the direction of the NIEHS and were conducted in compliance with NTP laboratory health and safety requirements and must meet or exceed all applicable federal, state, and local health and safety regulations. Animal care and use were in accordance with the Public Health Service Policy on Humane Care and Use of Animals. The prechronic and chronic studies were conducted in compliance with Food and Drug Administration (FDA) Good Laboratory Practice Regulations, and all aspects of the chronic studies were subjected to retrospective quality assurance audits before being presented for public review.

These studies are designed and conducted to characterize and evaluate the toxicologic potential, including carcinogenic activity, of selected chemicals in laboratory animals (usually two species, rats and mice). Chemicals selected for NTP toxicology and carcinogenesis studies are chosen primarily on the bases of human exposure, level of production, and chemical structure. The interpretive conclusions presented in this Technical Report are based only on the results of these NTP studies. Extrapolation of these results to other species and quantitative risk analyses for humans require wider analyses beyond the purview of these studies. Selection *per se* is not an indicator of a chemical's carcinogenic potential.

Listings of all published NTP reports and ongoing studies are available from NTP Central Data Management NIEHS, P.O. Box 12233, MD E1-02, Research Triangle Park, NC 27709 (919-541-3419). The Abstracts and other study information for 2-year studies are also available at the NTP's World Wide Web site: <http://ntp-server.niehs.nih.gov>.

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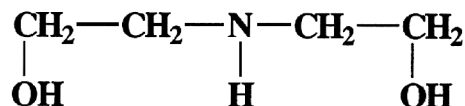
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ABSTRACT



DIETHANOLAMINE

CAS No. 111-42-2

Chemical Formula: C₄H₁₁NO Molecular Weight: 105.14

Synonyms: Bis-2-hydroxyethylamine; DEA; diethylolamine; 2,2'-dihydroxydiethylamine; diolamine; 2,2'-iminobisethanol; iminodiethanol; 2,2'-iminodiethanol

Diethanolamine is widely used in the preparation of diethanolamides and diethanolamine salts of long-chain fatty acids that are formulated into soaps and surfactants used in liquid laundry and dishwashing detergents, cosmetics, shampoos, and hair conditioners. Diethanolamine is also used in textile processing, in industrial gas purification to remove acid gases, as an anticorrosion agent in metalworking fluids, and in preparations of agricultural chemicals. Aqueous diethanolamine solutions are used as solvents for numerous drugs that are administered intravenously. Diethanolamine was selected for evaluation because its large-scale production and pattern of use indicate the potential for widespread human exposure. Male and female F344/N rats and B6C3F₁ mice received dermal applications of diethanolamine in 95% ethanol for 2 years. Genetic toxicology studies were performed in *Salmonella typhimurium*, L5178Y were performed in *Salmonella typhimurium*, L5178Y ovary cells, and B6C3F1 mouse peripheral blood erythrocytes.

RATS

Groups of 50 male rats were administered 0, 16, 32, or 64 mg diethanolamine/kg body weight in ethanol dermally for 2 years. Groups of 50 female

rats were administered 0, 8, 16, or 32 mg/kg in ethanol dermally for 2 years.

Survival, Body Weights, and Clinical Findings

Survival of vehicle control and dosed male and female rats was similar. Mean body weights of 64 mg/kg males were less than those of the vehicle controls beginning week 8, and mean body weights of females were generally similar to those of the vehicle control group. The only clinical finding attributed to diethanolamine administration was irritation of the skin at the site of application.

Pathology Findings

Minimal to mild nonneoplastic lesions occurred at the site of application in the epidermis of dosed male and female rats. The incidence of acanthosis in 64 mg/kg males, the incidences of hyperkeratosis in 32 and 64 mg/kg males and in all dosed female groups, and the incidences of exudate in 64 mg/kg males and in all dosed female groups were greater than those in the controls.

The incidences and severities of nephropathy were significantly increased in dosed female rats compared to the vehicle controls.

MICE

Groups of 50 male and 50 female mice were administered 0, 40, 80, or 160 mg diethanolamine/kg body weight in ethanol dermally for 2 years.

Survival, Body Weights, and Clinical Findings

Survival of dosed male groups was similar to that of the vehicle control group; survival of dosed female groups was significantly less than that of the vehicle control group. Mean body weights of 80 and 160 mg/kg males were less than those of the vehicle controls after weeks 88 and 77, respectively. Mean body weights of dosed groups of females were generally less than those of the vehicle controls during the second year of the study.

Pathology Findings

In male mice, the incidences of hepatocellular adenoma and of hepatocellular adenoma or carcinoma (combined) in all dosed groups and of hepatocellular carcinoma and hepatoblastoma in 80 and 160 mg/kg males were significantly increased compared to the vehicle controls. The incidences of hepatocellular neoplasms were significantly greater in dosed groups of female mice than in the vehicle control group. The incidences of hepatocellular neoplasms in all dosed groups of males and females exceeded the historical control ranges. Nonneoplastic hepatocyte changes were seen only in dosed male and female mice. Changes consisted of cytoplasmic alteration and syncytial alteration.

The incidences of renal tubule adenoma in males occurred with a positive trend; however, the incidences of carcinoma and hyperplasia did not follow this pattern. An extended evaluation of kidney step sections revealed additional adenomas and hyperplasias in all dosed groups. The combined analysis of single and step sections indicated a dose-related increase in the incidences of renal tubule hyperplasia and renal tubule adenoma or carcinoma (combined), and an increase in the incidences of renal tubule adenoma in male mice.

Incidences of thyroid gland follicular cell hyperplasia were increased in dosed male and female mice compared to vehicle controls.

Hyperkeratosis, acanthosis, and exudate were treatment-related changes in the skin at the site of application. The incidences of hyperkeratosis were significantly greater than those in the vehicle control groups in all dosed groups except 40 mg/kg females.

GENETIC TOXICOLOGY

Diethanolamine was not mutagenic in any of four strains of *Salmonella typhimurium*, in the presence or absence of S9 metabolic activation enzymes. No induction of trifluorothymidine resistance was observed in L5178Y mouse lymphoma cells treated with diethanolamine with or without S9. Diethanolamine did not induce significant sister chromatid exchanges or chromosomal aberrations in cultured Chinese hamster ovary cells, with or without S9. Peripheral blood samples collected from male and female mice exposed to 80 to 1,250 mg/kg diethanolamine dermally for 13 weeks showed no increase in micronucleated normochromatic erythrocytes.

CONCLUSIONS

Under the conditions of these 2-year dermal studies, there was *no evidence of carcinogenic activity** of diethanolamine in male F344/N rats administered 16, 32, or 64 mg/kg diethanolamine or in female F344/N rats administered 8, 16, or 32 mg/kg. There was *clear evidence of carcinogenic activity** of diethanolamine in male and female B6C3F₁ mice based on increased incidences of liver neoplasms in males and females and increased incidences of renal tubule neoplasms in males.

Dermal administration of diethanolamine to rats was associated with increased incidences of acanthosis (males only), hyperkeratosis, and exudate of the skin and increased incidences and severities of nephropathy in females. Dermal administration of diethanolamine to mice was associated with increased incidences of cytoplasmic alteration (males only) and syncytial alteration of the liver, renal tubule hyperplasia (males only), thyroid gland follicular cell hyperplasia, and hyperkeratosis of the skin.

* Explanation of Levels of Evidence of Carcinogenic Activity is on page 10. A summary of the Technical Reports Review Subcommittee comments and the public discussion on this Technical Report appears on page 12.

Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of Diethanolamine

	Male F344/N Rats	Female F344/N Rats	Male B6C3F ₁ Mice	Female B6C3F ₁ Mice
Doses in ethanol by dermal application	0, 16, 32, or 64 mg/kg	0, 8, 16, or 32 mg/kg	0, 40, 80, or 160 mg/kg	0, 40, 80, or 160 mg/kg
Body weights	64 mg/kg groups generally less than vehicle control groups	Dosed groups generally similar to vehicle control group	80 and 160 mg/kg groups less than vehicle control group	Dosed groups generally less than vehicle control group
Survival rates	14/50, 10/50, 21/50, 22/50	25/50, 29/50, 29/50, 24/50	40/50, 43/50, 34/50, 30/50	44/50, 33/50, 33/50, 23/50
Nonneoplastic effects	<u>Skin</u> : acanthosis (0/50, 2/50, 4/50, 10/50); hyperkeratosis (0/50, 3/50, 5/50, 11/50); exudate (0/50, 3/50, 2/50, 7/50)	<u>Skin</u> : hyperkeratosis (3/50, 13/50, 23/50, 23/50); exudate (1/50, 7/50, 7/50, 7/50) <u>Kidney</u> : nephropathy (40/50, 47/50, 48/50, 48/50); severity (1.2, 1.5, 1.9, 2.7)	<u>Liver</u> : cytoplasmic alteration (1/50, 17/50, 17/50, 12/50); syncytial alteration (0/50, 28/50, 38/50, 23/50) <u>Kidney</u> : renal tubule hyperplasia (standard and extended evaluation combined (3/50, 7/50, 7/50, 10/50) <u>Thyroid gland</u> : follicular cell hyperplasia (18/50, 22/49, 30/50, 42/50) <u>Skin</u> : hyperkeratosis (0/50, 13/50, 10/50, 17/50)	<u>Liver</u> : syncytial alteration (0/50, 2/50, 17/50, 18/50) <u>Thyroid gland</u> : follicular cell hyperplasia (18/50, 28/49, 32/50, 39/50) <u>Skin</u> : hyperkeratosis (1/50, 3/50, 8/50, 16/50)
Neoplastic effects	None	None	<u>Liver</u> : hepatocellular adenoma (31/50, 42/50, 49/50, 45/50); hepatocellular carcinoma (12/50, 17/50, 33/50, 34/50); hepatoblastoma (0/50, 2/50, 8/50, 5/50); hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma (39/50, 47/50, 50/50, 49/50) <u>Kidney</u> : adenoma (standard evaluation - 1/50, 4/50, 6/50, 6/50; standard and extended evaluation combined - 1/50, 6/50, 8/50, 7/50); adenoma or carcinoma (combined) (standard evaluation - 3/50, 5/50, 6/50, 8/50; standard and extended evaluation combined - 3/50, 7/50, 8/50, 9/50)	<u>Liver</u> : hepatocellular adenoma (32/50, 50/50, 48/50, 48/50); hepatocellular carcinoma (5/50, 19/50, 38/50, 42/50); hepatocellular adenoma or carcinoma (33/50, 50/50, 50/50, 50/50)

Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of Diethanolamine

	Male F344/N Rats	Female F344/N Rats	Male B6C3F ₁ Mice	Female B6C3F ₁ Mice
Level of evidence of carcinogenic activity	No evidence	No evidence	Clear evidence	Clear evidence
Genetic toxicology				
<i>Salmonella typhimurium</i> gene mutations:		Negative with and without S9 in strains TA98, TA100, TA1535, and TA1537		
Mouse lymphoma gene mutations:		Negative		
Sister chromatid exchanges				
Cultured Chinese hamster ovary cells <i>in vitro</i> :		Negative		
Chromosomal aberrations				
Cultured Chinese hamster ovary cells <i>in vitro</i> :		Negative		
Micronucleated erythrocytes				
Mouse peripheral blood <i>in vivo</i> :		Negative		

EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY

The National Toxicology Program describes the results of individual experiments on a chemical agent and notes the strength of the evidence for conclusions regarding each study. Negative results, in which the study animals do not have a greater incidence of neoplasia than control animals, do not necessarily mean that a chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of conditions. Positive results demonstrate that a chemical is carcinogenic for laboratory animals under the conditions of the study and indicate that exposure to the chemical has the potential for hazard to humans. Other organizations, such as the International Agency for Research on Cancer, assign a strength of evidence for conclusions based on an examination of all available evidence, including animal studies such as those conducted by the NTP, epidemiologic studies, and estimates of exposure. Thus, the actual determination of risk to humans from chemicals found to be carcinogenic in laboratory animals requires a wider analysis that extends beyond the purview of these studies.

Five categories of evidence of carcinogenic activity are used in the Technical Report series to summarize the strength of the evidence observed in each experiment: two categories for positive results (**clear evidence** and **some evidence**); one category for uncertain findings (**equivocal evidence**); one category for no observable effects (**no evidence**); and one category for experiments that cannot be evaluated because of major flaws (**inadequate study**). These categories of interpretative conclusions were first adopted in June 1983 and then revised in March 1986 for use in the Technical Report series to incorporate more specifically the concept of actual weight of evidence of carcinogenic activity. For each separate experiment (male rats, female rats, male mice, female mice), one of the following five categories is selected to describe the findings. These categories refer to the strength of the experimental evidence and not to potency or mechanism.

- **Clear evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a dose-related (i) increase of malignant neoplasms, (ii) increase of a combination of malignant and benign neoplasms, or (iii) marked increase of benign neoplasms if there is an indication from this or other studies of the ability of such tumors to progress to malignancy.
- **Some evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a chemical-related increased incidence of neoplasms (malignant, benign, or combined) in which the strength of the response is less than that required for clear evidence.
- **Equivocal evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a marginal increase of neoplasms that may be chemical related.
- **No evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing no chemical-related increases in malignant or benign neoplasms.
- **Inadequate study** of carcinogenic activity is demonstrated by studies that, because of major qualitative or quantitative limitations, cannot be interpreted as valid for showing either the presence or absence of carcinogenic activity.

When a conclusion statement for a particular experiment is selected, consideration must be given to key factors that would extend the actual boundary of an individual category of evidence. Such consideration should allow for incorporation of scientific experience and current understanding of long-term carcinogenesis studies in laboratory animals, especially for those evaluations that may be on the borderline between two adjacent levels. These considerations should include:

- adequacy of the experimental design and conduct;
- occurrence of common versus uncommon neoplasia;
- progression (or lack thereof) from benign to malignant neoplasia as well as from preneoplastic to neoplastic lesions;
- some benign neoplasms have the capacity to regress but others (of the same morphologic type) progress. At present, it is impossible to identify the difference. Therefore, where progression is known to be a possibility, the most prudent course is to assume that benign neoplasms of those types have the potential to become malignant;
- combining benign and malignant tumor incidence known or thought to represent stages of progression in the same organ or tissue;
- latency in tumor induction;
- multiplicity in site-specific neoplasia;
- metastases;
- supporting information from proliferative lesions (hyperplasia) in the same site of neoplasia or in other experiments (same lesion in another sex or species);
- presence or absence of dose relationships;
- statistical significance of the observed tumor increase;
- concurrent control tumor incidence as well as the historical control rate and variability for a specific neoplasm;
- survival-adjusted analyses and false positive or false negative concerns;
- structure-activity correlations; and
- in some cases, genetic toxicology.

NATIONAL TOXICOLOGY PROGRAM BOARD OF SCIENTIFIC COUNSELORS TECHNICAL REPORTS REVIEW SUBCOMMITTEE

The members of the Technical Reports Review Subcommittee who evaluated the draft NTP Technical Report on diethanolamine on 9 December 1997 are listed below. Subcommittee members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, subcommittee members have five major responsibilities in reviewing the NTP studies:

- to ascertain that all relevant literature data have been adequately cited and interpreted,
- to determine if the design and conditions of the NTP studies were appropriate,
- to ensure that the Technical Report presents the experimental results and conclusions fully and clearly,
- to judge the significance of the experimental results by scientific criteria, and
- to assess the evaluation of the evidence of carcinogenic activity and other observed toxic responses.

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SUMMARY OF TECHNICAL REPORTS REVIEW SUBCOMMITTEE COMMENTS

On 9 December 1997, the draft Technical Report on the toxicology and carcinogenicity studies of diethanolamine received public review by the National Toxicology Program's Board of Scientific Counselors' Technical Review Subcommittee. The review meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Dr. R.D. Irwin, NIEHS, introduced the toxicology and carcinogenesis studies of diethanolamine by discussing the uses and rationale for study, describing the experimental design, reporting on survival and body weight effects, and commenting on compound-related neoplastic and nonneoplastic lesions in rats and mice. The proposed conclusions for the 2-year studies were *no evidence of carcinogenic activity* in male and female F344/N rats and *clear evidence of carcinogenic activity* in male and female B6C3F₁ mice.

Dr. Goldsworthy, a principal reviewer, agreed with the proposed conclusions. He said that, because a majority of neoplasm responses observed in the companion studies of the fatty acid/diethanolamine condensates were concluded to result from the presence of free diethanolamine, some of his comments would also pertain to the condensates. Dr. Goldsworthy commented that the report should address if and how the distribution and metabolism of diethanolamine would be altered at various test concentrations and by the potential interactions with the different condensates. He said that, besides trying to link diethanolamine concentrations with neoplastic responses, it would be useful to chart comparative toxicities between the condensates and diethanolamine concentrations, as well as the potential for nitrosamine formation. Dr. Goldsworthy asked about the significance of the hepatoblastomas in treated male mice. Dr. J.R. Hailey, NIEHS, said that hepatoblastoma is a neoplasm with a fairly distinct morphology composed of primitive-appearing cells and appears to be part of the spectrum of the progression of liver neoplasms in the mouse; as such, with the higher background rate of liver neoplasms in mice, there is a concomitant increase in the incidence of hepatoblastoma.

Dr. Bailer, the second principal reviewer, agreed in principle with the proposed conclusions. He said the conclusions should be modified to note the significant negative trend in female rat mammary gland fibroadenomas and the increased survival experienced by rats administered diethanolamine. Dr. J.K. Haseman, NIEHS, said that a decrease in the incidence of mammary gland neoplasms is often associated with reduced body weight, although not in this case; therefore, more discussion might be merited. Dr. Bailer commented that the high liver neoplasm rates in control mice emphasize the importance of the concurrent controls in these studies, especially since the historical control database is so small for dermal studies using an ethanol vehicle.

Dr. Chatman, the third principal reviewer, did not agree with the conclusions for mice. She stated that diethanolamine is not a mutagen and is not metabolized to a reactive intermediate but can be converted to a carcinogenic nitrosamine. She felt that the potential for N-nitrosodiethanolamine formation should have been evaluated. Dr. Chatman referred to a letter received by the reviewers from the Alkanolamines Panel of the Chemical Manufacturers Association (CMA), which reported that rodent feed during some weeks of the studies was contaminated with high bacterial counts. She thought this could have enhanced N-nitroso-diethanolamine formation. Dr. Irwin responded that published studies with N-nitrosodiethanolamine given in drinking water show it to be a potent liver carcinogen in F344/N rats but a noncarcinogen in B6C3F₁ mice.

There were questions about the possible impact of *Helicobacter hepaticus* on the incidence of liver neoplasms in mice. Dr. Hailey said that in frozen tissues from about 20 animals, 10 males and 10 females, polymerase chain reaction analysis for *H. hepaticus* was negative. Dr. Goldsworthy asked for comment on the impact of increased liver neoplasm rates in control mice relative to interpretation of bioassay results. Dr. Hailey replied that, in view of higher background incidence, other components have to be assessed, especially progression to a malignant state and increases in numbers or multiplicity; both were

dramatically increased in these studies. Dr. Hecht agreed that formation of nitrosamines was not likely, but he was disappointed with the lack of detail in the analytical methods description so that contamination of diethanolamine with N-nitroso-diethanolamine could not be ruled out. Dr. Irwin said he would increase the detail in the analytical methods. Dr. G.N. Rao, NIEHS, stated that standards for the NIH-07 diet used since 1984 are much more stringent than those of most commercially available diets with regard to allowable bacterial counts.

Dr. W. Stott, Dow Chemical Company, representing the Alkanolamines Panel of the CMA, said that their major concerns with the study were questions about technical aspects of the bioassay and the inconsistency between the genotoxicity and carcinogenicity findings. Among technical questions which he thought should have been better discussed in the report were the

choice of a dermal rather than an oral route of administration, the use of an ethanol vehicle, which has potential promotional/carcinogenic effects in itself, the potential for nitrosamine formation *in vivo*, and high liver neoplasm incidence in control mice. Dr. Stott reported that the Alkanolamines Panel plans to conduct mechanistic studies to help understand the NTP mouse bioassay results and their relevance to humans.

Dr. Goldsworthy moved that the Technical Report on diethanolamine be accepted with the revisions discussed and the conclusions as written for male and female rats, *no evidence of carcinogenic activity*, and for male and female mice, *clear evidence of carcinogenic activity*. Dr. Bailer seconded the motion, which was accepted with six yes votes to one no vote (Dr. Chatman) and one abstention (Dr. Bus).